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Quantitative capillary electrophoresis and its application to the polyanionic quinobene*

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Abstract: Factors affecting the accuracy of capillary electrophoresis (CE) assays in general are discussed. Methods to improve the reproducibility and reliability of these assays are suggested. The improvements are demonstrated by developed CE assays for quinobene and suramin. The assays were reproducible (RSD <2%), accurate (error <2%), and linear over a concentration range of 1–800 μg ml^{-1} (r^2 = 0.999).

Keywords: *Capillary electrophoresis; accurate assay; quinobene; suramin; polyanions.*

Introduction

Capillary electrophoresis (CE) is an extremely efficient separation technique producing several hundred thousand theoretical plates per metre [1, 2]. The speed, ease of operation, and ability to work with ionic organics make CE a valuable technique in pharmaceutical analysis. The principle, instrumentation and application of CE have been reviewed recently [3, 4]. Poor precision (high RSD values) in peak area quantitation has generally limited its use to qualitative analysis. In the few published quantitative applications, the RSD of peak area averaged about 5% [5–11]. The RSD of one assay was 12% [5]. In the reports where internal standards were used, the RSD values were 2–3% [9, 11]. However, the ruggedness and accuracy of the assays were not stated.

Quantitative aspects of CE were recently reviewed by Goodall *et al.* [12]. Modern CE instruments equipped with automatic sample loader and capillary temperature control have reduced RSD values of peak area to adequate level, but the robustness and absolute quantitative capabilities of CE have yet to be demonstrated. Stevenson [13] suggested that progress in quantitative application of CE was slow and limited because factors affecting the reliability of CE assays were not fully understood. This paper presents a review on and an examination of factors that affect the precision and accuracy of CE assays. The authors offer suggestions and strategies for accurate CE assays and present the successful development of a reliable and rugged CE assay for the polyanionic quinobene.

Quinobene (S, Fig. 1), the tetrasodium salt of 4,4′-bis(8-hydroxy-5-sulpho-7-quinoline-azo)-stilbene-2,2′-disulphonic acid, is a member of the group of large polyanions that inhibit HIV replication [14]. It is prepared by coupling 8-hydroxyquinoline-5-sulphonic acid with the diazonium salt of 4,4′-diaminostilbene-2,2′-disulphonic acid.

Experimental

Reagents and materials

Quinobene (S), lots LK-17-17-3, LK-17-24-1, 873.A.91.2 and 873.A.92.301 were received from the US National Cancer Institute. Lot LK-17-17-3 was used in the method development and as the reference standard. It was characterized by elemental, spectral and chromatographic data as 80.4% quinobene. The remainder of the sample was 1.8% UV-absorbing organic impurities, 2.6% excess sodium ion and 15.2% H_2O. Sulphanilic acid, the internal standard (IS), was purchased from Eastman Organic Chemicals (Rochester, NY). All chemicals were used without further purification. Sample solutions were prepared by dissolving weighed amounts of S in the internal standard solution (40 μg IS ml^{-1} distilled water).

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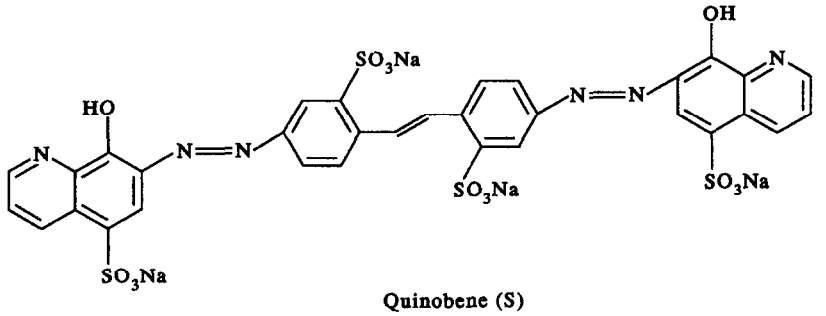
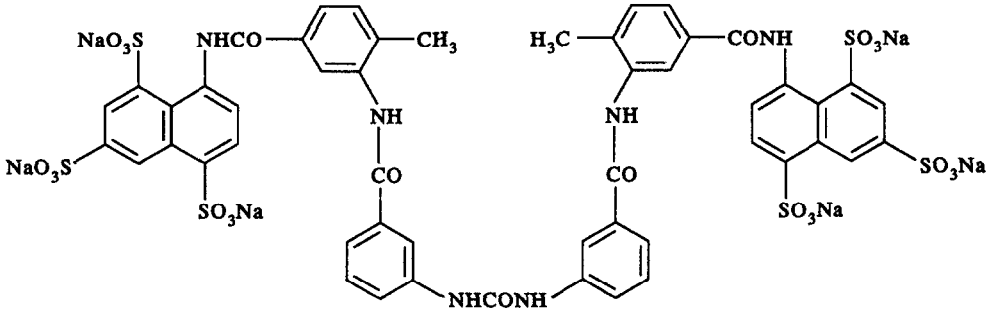
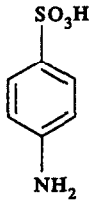


Quinobene (S)



Suramin



Sulphanilic acid (IS)

Figure 1
Molecular structure of quinobene (S), sulphanilic acid (IS) and suramin.

Tris (hydroxymethyl)aminomethane (TRIS) and boric acid were purchased from Mallinkrodt (Paris, KY). Ethylene-diaminetetraacetic acid, disodium salt, dihydrate (EDTA) was from Aldrich (Milwaukee, WI). Polyethylene glycol (PEG) linear polymers were from Fluka (Ronokonkoma, NY). The chemicals were reagent grade. Buffer solutions were prepared with distilled water.

Capillary electrophoresis (CE)

CE was performed on a Biofocus 3000 Electrophoresis System using a 36 cm × 50 μm, coated glass capillaries (both from BIO-RAD, Hercules, CA). The run buffer was Tris-boric acid (pH 8.6; 300 mM) containing 2 mM EDTA and 4% each of 6K, 12K, 20K and 35K PEG. Loading was done electrophoretically at 12 kV for 6 s or hydrodynamically (pressure) at 120 psi.s. The run voltage was 15 kV. The analyte ions migrated, counter to electroosmosis, from the negative to the positive electrode. Detection by UV at 254 nm was at the positive electrode. Data were collected and processed with the Biofocus 3000 Integration system (BIO-RAD, Hercules, CA).

Results and Discussion

The accuracy of a separation-based assay depends on the linear proportionality of peak

intensity versus sample quantity loaded. In addition to the analyte concentration, peak intensity in CE is a function of zone diffusion and migration time of the analyte. Thus, an accurate CE assay requires precise sample loading, reproducible zone broadening during migration, precise migration time, and proper peak signal integration.

Sample loading

Because automatic sample loading is more precise than manual loading [8, 15], a BIO-RAD Biofocus 3000 automatic CE instrument was used in this study. Sample loading can be achieved electrophoretically or hydrodynamically. Table 1 indicates that electrophoretic loading is very sensitive to the ionic concentration of sample solutions. In these solutions, S varied from 100 to 2100 μg while IS was at 40 μg ml^{-1}. Total ionic concentration in these solutions was 0.6–9.2 mM. The normalized peak area of S and IS decreased markedly with increase in the ionic strength of sample solutions. The increase in ionic strength enhanced the conductivity of the sample solution which resulted in reduced electrostacking and sample loading [16]. The reduction in loading appeared universal to all analyte ions, as the S/IS peak area ratio remained relatively close (RSD = 13.7%). Hydrodynamic loading was far more precise. In this case, the RSD values of the peak area of S, IS and their ratio were 3–4%. Since ionic concentrations of standard solutions and test solutions were often different, to avoid variance caused by electrostacking, hydrodynamic loading should always be used in an assay.

When the capillary inlet is transferred in and out of the sample solution and the buffer reservoir, diffusion transport and inadvertent hydrodynamic flow at the solution boundaries are known to cause loading variation [17]. Use of a long loading zone or gel-filled capillary has been suggested to minimize the variation. Long loading zone leads to zone broadening and reduced resolution [16]. A gel-filled capillary is not practical when washing and refilling of the capillary between runs is required. As a compromise, a viscous buffer containing non gel linear polymers (4% each of 6K, 12K, 20K and 35K PEG) was used in this study to minimize solution boundary problems. Data in Table 2 were obtained from electrophoretic loading of two identical sample solutions (100 μg S plus 40 μg IS ml^{-1} water). Each solution was consecutively electrophoresed six times. Electrophoretic loading was used to detect solution boundary problems. When these exist, the ionic concentration of the sample solution will increase after each loading, due to diffusion transport or hydrodynamic flow of the high ionic buffer (300 mM) into the low ionic sample solution (0.6 mM). This will result in reduction in subsequent loading because of decrease in electrostacking. Data in Table 2 indicate that solution boundary problems still exist when buffer containing viscous non gel PEG was used. For each

Table 1
Comparison of electrophoretic and hydrodynamic loading

Run	Conc.†	Electrophoretic loading*			Hydrodynamic loading*		
		S‡	IS	S‡/IS	S‡	IS	S‡/IS
1	109.0	64935	2744	23.46	32799	2067	15.86
2	212.1	37954	1665	22.79	32687	2101	15.55
3	398.7	20383	948	21.50	34976	2185	16.01
4	615.4	13554	712	19.03	33947	2099	16.17
5	807.0	14898	726	20.52	35437	2161	16.39
6	979.3	11444	613	18.67	35396	2186	16.19
7	1211.4	8991	490	18.34	34697	2125	16.32
8	1419.4	6917	450	15.37	35888	2281	15.73
9	1601.0	9357	500	18.36	34768	2226	15.62
10	1825.9	13140	559	23.40	35605	2247	15.84
11	2084.9	12750	529	24.10	34388	2365	14.54
	Mean	18484	903	20.52	34599	2186	15.84
	RSD (%)	88.2	77.7	13.7	3.1	4.1	3.2

See text for CE conditions.
* Peak area count.
† μg of S (quinobene) ml^{-1} of IS solution (40 μg sulphanilic acid ml^{-1} H_2O).
‡ Normalized for S concentration.

Table 2
The effect of solution boundary problems on loading problems on loading precision

Run	Sample	Area			Height			Migration time (min)		
		S	IS	R_A*	S	IS	R_H*	S	IS	R_t*
1	1	2983	1359	2.19	2253	1131	1.99	42.47	20.85	2.04
2	1	2510	1340	1.87	2064	1124	1.84	42.73	20.99	2.04
3	1	2181	1319	1.64	1884	1117	1.69	43.38	21.29	2.04
4	1	2389	1379	1.73	1976	1113	1.78	43.98	21.50	2.05
5	1	1890	1251	1.51	1708	1056	1.62	44.50	21.71	2.05
6	1	2092	1339	1.56	1835	1063	1.73	45.08	22.00	2.05
7	2	2809	1338	2.10	2198	1074	2.05	45.22	22.07	2.05
8	2	2370	1298	1.83	1954	1053	1.86	45.84	22.25	2.06
9	2	2198	1295	1.70	1877	1051	1.79	46.65	22.65	2.06
10	2	2241	1327	1.69	1882	1055	1.78	47.33	22.88	2.06
11	2	2167	1305	1.66	1837	1029	1.79	47.99	23.20	2.07
12	2	2350	1386	1.70	1930	1071	1.80	48.14	23.22	2.07

See text for CE conditions. Samples were prepared by dissolving 1 mg S in 10 ml water solution containing 0.4 mg IS.
* S/IS ratio.

sample solution, the S peak decreased significantly after each loading during the first two or three loadings and remained steady thereafter. The faster migrating IS peak remained relatively constant in all six loadings. The preferential decrease of the much slower S peak suggested that inadvertent hydrodynamic flow was a more serious problem than transport diffusion. To avoid the effect of hydrodynamic flow, fresh aliquots of sample solutions should be used for each loading in a CE assay. The amount of sample solutions in CE autosampler vials is usually small, evaporation of sample solution is a well recognized problem leading to loading error. To eradicate this and the diffusion transport problems, use of an internal standard is suggested.

Zone broadening and migration time

As the analyte zone moves along the capillary, zone broadening takes place. Zone broadening is a function of amount of sample loaded, ionic density gradient in the buffer after sample loading, sample ion concentration in the zone due to molecular migration, capillary temperature, electrophoretic migration and electroosmosis [1, 18]. The latter two, which contribute to migration time are, in turn, affected by the run voltage, run buffer and the inner wall of the capillary. Therefore, to ensure reproducible zone broadening and precise migration time of analytes, the variance of each of the above-mentioned attributes has to be minimized.

The loading amount, run voltage and capillary temperature are precisely controlled by automatic instruments. The temperature gradient inside the capillary is negligible when the internal diameter of the capillary is <100 μM [18]. Washing and refilling the capillary with fresh run buffer before each electrophoresis maintains constancy in the run buffer, and hopefully the inner wall of the capillary. Foret *et al.* [1] demonstrated that variance in the electrophoretic mobility of the analyte was insignificant if the ionic concentration of the sample was less than 1% of that of the buffer. These precautions taken, an RSD of $<1\%$ was achieved for migration time, however, the RSD of the peak area was still 2–10% [19]. To further improve the peak area precision, we sought to minimize electroosmosis (EOF) by using a polymer-coated capillary. Table 3 presents precision data obtained with the coated capillary. The capillary was washed and refilled before each electrophoresis run, with the PEG-containing buffer. Six aliquots of a single sample solution were each electrophoresed in triplicates. RSD of the peak area (10%) was still three times that of the migration time. The peak intensities and the migration time increased gradually after each electrophoresis run. Linear regression analysis of the migration time vs run number gave linear equations of $y = 0.24x + 33.44$ and $y = 0.10x + 16.48$, respectively, for S and IS. The correlation coefficients (r) are both 0.995. The changes in peak area and peak height with run number were more random and gave the following linear equations:

Table 3
The precision of area, height and migration time (min) of S and IS under triplicate hydrodynamic loading of six samplings of a single solution

		Area			Height			Migration time		
Run	Sample	S	IS	R_A*	S	IS	R_H*	S	IS	R_t*
1	1	2599	1480	1.756	22831	13188	1.731	33.81	16.64	2.031
2	1	2465	1438	1.714	22001	12936	1.700	33.92	16.68	2.033
3	1	2522	1477	1.707	22317	13221	1.687	33.98	16.71	2.033
4	2	2478	1475	1.680	21786	13021	1.673	34.13	16.79	2.032
5	2	2646	1490	1.775	22762	13089	1.739	34.49	16.86	2.045
6	2	2682	1532	1.750	23099	13295	1.737	34.89	17.05	2.046
7	3	2773	1547	1.792	23396	13217	1.770	35.28	17.25	2.045
8	3	2687	1515	1.773	22944	13055	1.757	35.47	17.29	2.051
9	3	2719	1555	1.748	23009	13226	1.739	35.65	17.36	2.053
10	4	2743	1560	1.758	23001	13127	1.752	35.97	17.52	2.053
11	4	2379	1428	1.665	21169	12386	1.709	36.09	17.54	2.057
12	4	2910	1639	1.775	23987	13481	1.779	36.38	17.66	2.060
13	5	2784	1665	1.672	23295	13542	1.720	36.53	17.76	2.056
14	5	2958	1662	1.779	24207	13506	1.792	36.77	17.82	2.063
15	5	2905	1648	1.762	24020	13378	1.795	36.92	17.88	2.064
16	6	2902	1585	1.830	23760	12985	1.829	37.23	18.02	2.066
17	6	3474	1869	1.858	26605	14292	1.861	37.49	18.11	2.070
18	6	3425	1955	1.751	26346	14615	1.802	37.57	18.16	2.068
	RSD (%)	10.3	8.6	2.8	5.8	3.6	2.7	3.5	2.9	0.6

See text for CE conditions. Sample solution was prepared by dissolving 1 mg S in 10 ml water containing 0.4 mg IS.
* Ratio of S/IS values.

Run number vs S area: $y = 43.47x + 2367.6$, $r = 0.790$,
Run number vs IS area: $y = 21.19x + 1383.2$, $r = 0.803$,
Run number vs S height: $y = 190x + 21557$, $r = 0.734$,
Run number vs IS height: $y = 52.99x + 12802$, $r = 0.570$.

Examination of the slope/intercept ratio, an indication of change per electrophoresis run, revealed that the increases for peak height and migration time are similar (0.4–0.9% run^{-1}). The incrases for peak area (1.5–1.8% run^{-1}) are 2–3 times those of migration time. In addition, the increases in area, height and migration time are consistently larger for the slower S than the faster IS peak. These observations suggest that a significant portion of the increases in area and height is systematic and is caused by the increase in migration time. This is also evident from the much smaller RSD of the S/IS ratio of area, height and migration time (R_A, R_H and R_t, respectively) than those of individual peaks. Similar results were obtained from experiments with single or triplicate electrophoresis runs of seven different sample solutions. Table 4 summarizes the RSDs of R_A, R_H and R_t from those experiments. The findings are similar to those of Weiss *et al.* [9] where an uncoated capillary was used and EOF was not curtailed. Evidently, minimizing EOF with coated capillary failed to create a constant capillary inner wall or a constant EOF. Small amounts of anionic organic analytes might have adsorbed irreversibly onto the polymer-coated inner wall of the capillary during each electrophoresis. The adsorbed organics were not completely removed by the washing and refilling cycle. Thus, EOF increased with each electrophoresis run due to the ever-increasing amount of anionic organics adsorbed onto the inner wall. Since movement of the analyte ions (towards the positive electrode) was counter to EOF, the mobility of the analytes decreased or their migration time increased with each electrophoresis run. This migration time change created a bias in peak detection [20]. The adsorbed organics appeared removable by washing and storing water inside the capillary for at least 4 h. After this treatment, the migration times of the analytes returned to their initial values again. However, this capillary treatment would not be practical for an assay. Since this migration time change due to adsorbed organics was systemic to all analyte peaks within the sample, a more practical way to treat this systematic error is to employ an IS. Ideally, the IS should have a migration time similar to that of the analyte.

Table 4
The mean and precision (RSD) of R_A, R_H and R_t

Data description	R_A		R_H		R_t	
	Mean	RSD (%)	Mean	RSD (%)	Mean	RSD (%)
(A) From triplicate run of six samplings of a single sample solution						
1 All 18 runs	1.753	2.8	1.754	2.7	2.051	0.6
2 Triplicate of sample 1	1.725	1.2	1.706	1.1	2.032	0.0
3 Triplicate of sample 2	1.735	2.3	1.716	1.8	2.041	0.3
4 Triplicate of sample 3	1.771	1.0	1.755	0.7	2.050	0.2
5 Triplicate of sample 4	1.733	2.8	1.746	1.7	2.056	0.1
6 Triplicate of sample 5	1.738	2.7	1.769	2.0	2.061	0.2
7 Triplicate of sample 6	1.813	2.5	1.831	1.3	2.068	0.1
8 Means of six samples	1.753	1.9	1.754	2.5	2.051	0.6
(B) From triplicate run of single sampling of seven sample solutions						
1 All 21 runs	1.710	2.4	1.709	2.2	2.056	0.5
2 Triplicate of sample 1	1.675	0.3	1.642	0.3	2.039	0.2
3 Triplicate of sample 2	1.765	1.1	1.748	0.9	2.045	0.2
4 Triplicate of sample 3	1.748	1.6	1.720	0.7	2.050	0.2
5 Triplicate of sample 4	1.680	0.6	1.683	0.6	2.057	0.2
6 Triplicate of sample 5	1.697	2.2	1.720	1.4	2.062	0.1
7 Triplicate of sample 6	1.695	2.4	1.708	1.5	2.066	0.2
8 Triplicate of sample 7	1.712	0.9	1.742	0.3	2.069	0.2
9 Means of seven samples	1.710	1.8	1.709	2.0	2.055	0.5
(C) From single run of single sample of seven sample solutions						
1 All seven runs	1.702	2.2	1.697	1.7	2.020	0.3

See text for CE conditions. R_A and R_H were normalized for S concentration. Sample solution in Part A was prepared by dissolving 1.0 mg S in 10.0 ml IS solution. Sample solutions in Part B and C were prepared by dissolving 0.98–1.04 mg of S in 10.0 ml IS solution. IS solution was prepared by dissolving 10 mg IS in 25.0 ml water.

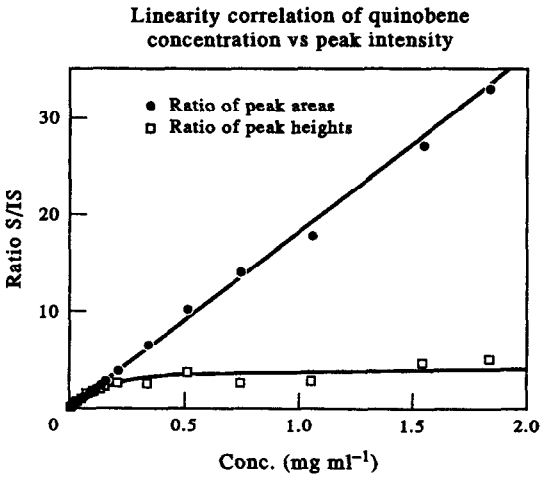


Figure 2
Linear correlation of R_A and R_H versus quinobene concentration. R_A and R_H are the area and height ratio of quinobene/internal standard peaks, respectively.

As shown in Tables 3 and 4, the RSD of R_A and R_H are consistently larger than that of R_t. This suggests that factors other than migration time also contribute to zone broadening. As the sample is loaded and the analytes travel across the supposedly static buffer inside the capillary, the ionic density of the buffer is perturbed. The variance in this pertubation of ionic density gradient in the buffer after sample loading and during molecular migration contributes to the variance of zone broadening. The variance is more serious for slow migrating zones than for fast migrating ones, and is probably responsible for the larger RSDs of R_A and R_H. This variance is random and should improve with multiple run averaging. Indeed, as shown in Table 4, Part A, when the precision was recalculated from averages of triplicates, the RSDs of R_A and R_H were reduced to 1.9 from 2.8% (Table 3). The RSD of R_t, which is not affected by this random variance, remained the same (0.6%).

Peak detection

When the sample solution concentration range is narrow, as data from Tables 3 and 4 indicate, both peak area and peak height ratios are similar in precision. However, Moring *et al.* [19] suggested that peak height in CE detection was less accurate than peak area. At high analyte concentration, peak distortion occurred due to the small i.d. of the capillary and led to distortion of linearity. Therefore, the effect of the peak distortion on the S/IS peak ratios at higher and wider sample (S)

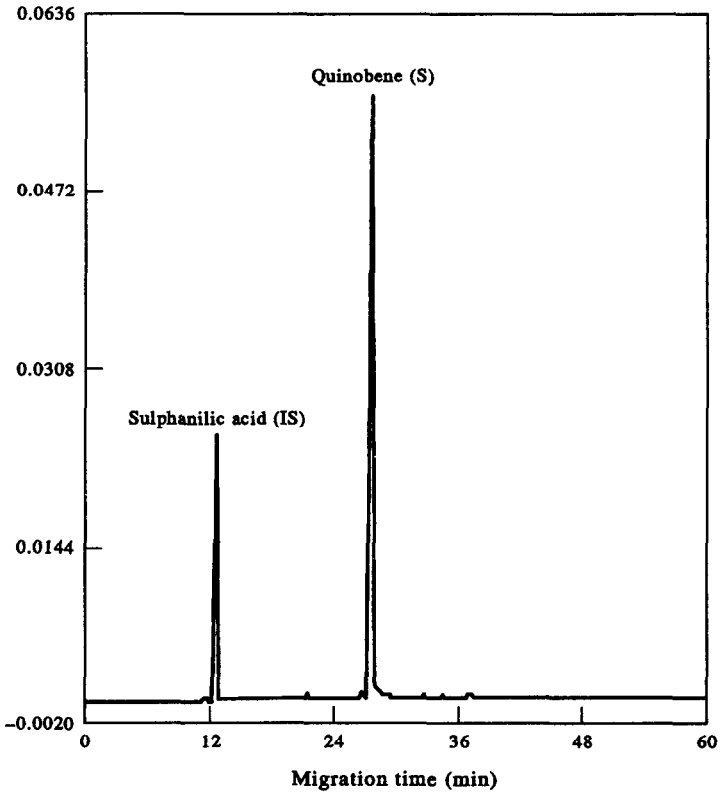


Figure 3
Typical electropherogram of a water solution of quinobene (S, 100 μg ml^{-1}) and sulphanilic acid (IS, 40 μg ml^{-1}). See text for CE conditions. Loading: hydrodynamic, 120 psi.s (about 10 nl). Capillary: 36 cm × 50 μm, coated. Run voltage: 15 kV.

concentration range was also investigated. Table 5 shows that, at an S concentration range of 60–180 μg ml^{-1} IS solution (40 μg IS ml^{-1} water), both R_A and R_H are linear with the S concentration. The accuracy of the R_A data is slightly better. The day-to-day reproducibility of linearity and accuracy or ruggedness of the assay (Table 6) definitely favours R_A. Over a wider range of S concentrations (1–2000 μg ml^{-1}), while R_A was linear for the entire range, R_H was linear only to 150 μg ml^{-1} (Fig. 2). Although the advantage of peak area measurement over peak height measurement in CE quantitation is widely accepted, it has not been substantiated by published work.

CE assay for quinobene

Based on the above discussion, a CE assay was developed for quinobene which exhibited an anti-HIV activity [21]. The development and the specificity of the CE separation is presented in a separate paper [22]. To maximize the accuracy of the assay, an automatic CE system with hydrodynamic loading, a viscous buffer containing molecular sieving PEG, a coated capillary which was washed with water and refilled with the buffer before each electrophoresis, an IS, and peak area ratio of S/IS were used. A typical electropherogram is presented in Fig. 3.

The precision of the method was measured by the RSD of the S/IS peak area ratio (R_A), normalized for S concentration. Based on six samplings of a single standard solution (100 μg S plus 40 μg IS ml^{-1} water) or one sampling each of seven standard solutions (98–106 μg S plus 40 μg IS ml^{-1} water), the RSD was 1.9% (Table 4). Each sampling was electrophoresed three times. Based on peak area data from six standard solutions of 60–180 μg S plus 40 μg IS ml^{-1} water (Table 5), the CE assay was linear ($y = 0.01601x + 0.131$, $r = 0.9996$) and accurate (1.1% error). When the S concentration range was extended from 1 to 2000 μg ml^{-1}, the linearity was still excellent ($y = 1.7693x + 0.0984$, $r = 0.9992$). Based on a 3:1 signal-to-noise ratio, the observed lower limit of quantitation (LOQ) for quinobene was 1 μg ml^{-1}. The day-to-day CE assay results

Table 5
Linearity and accuracy of the CE assay for quinobene (S) in IS solution

Sample	Peak area S	Peak area IS	R_A*	μg S ml^{-1} IS solution Actual	Found†	Error‡ (%)
1	1734	1571	1.105	59.1	60.8	2.9
2	2136	1502	1.422	82.1	80.6	1.8
3	2475	1425	1.736	100.3	100.3	0.1
4	3002	1422	2.111	124.7	123.7	0.9
5	3610	1407	2.565	152.6	152.1	0.3
6	4058	1380	2.940	174.2	175.5	0.7
					Mean	1.1
Sample	Peak height S	Peak height IS	R_H*	μg S ml^{-1} IS solution Actual	Found§	Error‡ (%)
1	17136	13797	1.242	59.1	55.9	5.5
2	19820	13287	1.491	82.1	82.0	0.1
3	21861	12860	1.699	100.3	103.8	3.4
4	24559	12702	1.925	124.7	127.4	2.1
5	27358	12556	2.178	152.6	153.9	0.9
6	29215	12491	2.338	174.2	170.7	6.4
					Mean	3.1

See text for CE conditions. Linear regression analysis of R_A (y) vs actual S concentration (x) gave $y = 0.01601x + 0.131$, $r = 0.9996$. Linear regression analysis of R_H (y) vs actual S concentration (x) gave $y = 0.00955x + 0.708$, $r = 0.9975$.

*Ratio = S/IS.

†Found = $(R_A - 0.131)/0.01601$.

‡Error = (actual − found)/actual.

§Found = $(R_H - 0.788)/0.00955$.

Table 6
Day-to-day variation of linearity and accuracy of the CE assay

Date	Linearity (r) R_A	Linearity (r) R_H	Accuracy (error)* R_A	Accuracy (error)* R_H	n
7/92	0.9995	0.9985	0.6	1.8	6
8/92	0.9995	0.9975	1.6	4.1	6
8/92	0.9985	0.9995	2.7	0.9	6
9/92	0.9975	0.9659	1.4	12.0	6
10/92	0.9995	0.9892	2.8	18.6	10
Mean	0.9989	0.9901	1.8	7.5	

*Mean error within each data set.

were consistent and compared favourably with those of HPLC assays [22], confirming the ruggedness and reliability of the CE assay.

When this CE assay was applied to another polysulphonated organic compound, suramin (Fig. 1), similar results were obtained. Based on single electrophoresis run of six samplings of a single standard solution or single samplings of five standard solutions (40 μg suramin plus 100 μg IS ml^{-1} water), the RSD of R_A was 1.8%. Based on six standard solutions of 10–60 μg suramin plus 100 μg IS ml^{-1} water, the CE assay for suramin was linear ($y = 0.736x + 0.630$, $r = 0.9998$) and accurate (1.5% error). The assay remained linear ($y = 1.472x + 0.368$, $r = 0.9990$) for the suramin concentration range of 0.4–800 μg suramin ml^{-1} IS solution. Similar to that observed for quinobene (Fig. 2), the ratio of peak area remained linear for the entire concentration range while the peak height ratio was linear for the low concentration range only.

Conclusion

Due to poor precision in peak area, the use of CE for quantitative analysis has been limited. Many factors, particularly analyte zone broadening during migration, affect the accuracy of the peak area. By understanding these factors, experiments can be designed to overcome these problems. Random errors can be reduced with automatic instruments and averaging of multiple runs. Systematic errors can be eliminated with an appropriate internal standard. By this approach, reliable and accurate CE assays have been developed for organic polyanions — quinobene and suramin. Both assays are linear ($r > 0.999$), precise (RSD = 1.8%) and accurate (error <1.5%).

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